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Key factors affecting seed germination of *Copaifera langsdorffii*, a Neotropical tree

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ABSTRACT

In natural conditions biotic and abiotic factors interact, synergistically affecting seed germination. In this study, we experimentally simulated natural conditions that occur during seed dispersal that can affect the germination of *Copaifera langsdorffii*. Specifically we evaluated the effect of aril removal by different dispersal agents (birds and ants) and fire on germination. The seeds were submitted to the following treatments: Control (seeds placed to germinate with aril intact); Acid (simulation of passage through the digestive tract of a bird); Aril removal (simulation of aril removed by ants); Fire (seeds exposed to fire). Germination percentage and time varied among treatments (X^2=89.735, P<0.001; X^2=16.225, P<0.001, respectively). None of the control seeds (intact aril) germinated. Treatments that simulated dispersal (Acid, Aril removal) did not differ in germination percentage, with about 50% of the seeds germinating, however, the acid treatment accelerated seed germination. Fire also had a positive effect on seed germination with about 80% of the seeds germinating. Our results demonstrate the importance of dispersal agents to the population dynamics of *C. langsdorffii*. Furthermore, the capacity of seeds of *C. langsdorffii* to tolerate high temperatures is an important attribute for the occurrence of this species in the Cerrado.

Keywords: Cerrado, *Copaifera langsdorffii*, fire, plant-disperser interaction, seed germination

Introduction

Seed germination is a critical phase of the plant life cycle, influencing the distribution and abundance of species in plant communities (Wulff 1986; Armstrong & Westoby 1993). Biotic factors, intrinsic to the seed and/or interactions with other organisms and abiotic factors, such as light, temperature, humidity and fire, affect germination differently (Baskin & Baskin 1998). Zoochorous seeds have fleshy structures that attract and reward their dispersers (Christianini *et al.* 2007) and often have substances that inhibit germination (Cipollini & Levey 1997; Yagihashi & Miyamoto 1998; Robertson *et al.* 2006). Thus, in addition to transporting seeds away from the mother plant, seed dispersers can be important to the successful germination of some species by removing compounds that inhibit germination (Robertson *et al.* 2006; Silveira *et al.* 2012; Lessa *et al.* 2013). Birds and ants are important groups that mutually interact as seed dispersers and can remove inhibitors, thus promoting germination (Meyer & Witmer 1998; Christianini & Oliveira 2010; Guerta *et al.* 2011; Lima *et al.* 2013), however, this influence is not uniform among zoochorous species (Barnea *et al.* 1991; Figueroa & Castro 2002).

Fire can interfere in many aspects of plant development, especially in the biology of seeds (Paula *et al.* 2009). The effects of fire on the seeds include loss of viability (Schmidt *et al.* 2005), dormancy break (Ribeiro *et al.* 2013), and the activation of genes important to germination by the presence of smoke (Moreira *et al.* 2010). These effects depend mainly on the degree of tolerance a seed and the species life history has to high temperatures (Luna *et al.* 2007). The Cerrado is an environment in which fire has been a recurrent factor for thousands of years (Salgado-Laboriau *et al.* 1997). In fact, recent studies have shown that seeds of plants of the Cerrado tend to be more tolerant to high temperatures than seeds of forest plants (Ribeiro *et al.* 2013; Ribeiro & Borghetti 2014). Despite these recent efforts, studies assessing the effects of fire on the germination of native Cerrado plant species remain scarce, especially investigations involving species that are not endemic to this biome.

Copaifera langsdorffii (Fabaceae) is a species of tropical tree of 7-30 m in height (Carvalho 2003). The species is widely distributed in South America (Carvalho 2003). In Brazil the species occurs in the physiognomies of Cerrado, Atlantic Forest and gallery forest, from the north to the south (Almeida *et al.* 1998). *Copaifera langsdorffii* presents supra-annual fruiting, with alternating years of high and low or no fruit production (Pedroni *et al.* 2002; Fagundes *et al.* 2013). Flowering occurs from November to January

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and fruits mature in July to September, coinciding with the period of greatest deciduousness (Pedroni *et al.* 2002; Fagundes *et al.* 2013). There are many types of dispersers of the seed of *Copaifera langsdorffii*, but the primary seed dispersers are birds (see Rabello *et al.* 2010), and the secondary dispersers are ants (see Leal & Oliveira 1998; Silva & Souza 2014). The seeds of *C. langsdorffii* have orthodox behavior (Bezerra *et al.* 2002) and pre-germination treatments of scarification can accelerate the germination process (Perez & Prado 1993). In addition, Souza & Fagundes (2014) showed that seed size as key factor in germination of *C. langsdorffii*.

In natural conditions, biotic and abiotic factors interact synergistically directly affecting time to germination and the percentage of successful seed germinations. Thus, the objective of this study was to evaluate seed dispersal of *C. langsdorffii*, in laboratory situations simulating field conditions, in order to determine the effects of biotic and abiotic factors on the process of seed germination. Specifically we seek to answer the following questions: (i) What influence does aril removal have on seed germination of *C. langsdorffii*? (ii) Knowing that birds and ants can disperse the seeds of *C. langsdorffii* and that they remove the aril differently, do they have differing affects on germination? (iii) Does fire affect seed germination of *C. langsdorffii*?

Materials and methods

Study area

Fieldwork was conducted in a Cerrado (Brazilian savanna) area located in the Floresta Nacional de Paraopeba (FLONA-PARAOPEBA, 19°20'S, 44°24'W), in the municipality of Paraopeba, in southeastern Brazil. The climate is type AW according to the Köppen classification, with a rainy summer and a dry season from April to September, corresponding to the fall and winter. The average annual temperature is 20ºC and the annual accumulated rainfall is about 1300 mm (INMET 2015). Annual climatic variation is shown in Fig. 1.

Data collection

In August 2013, 10 reproductive individuals of *Copaifera langsdorffii* Desf. were selected at the study area. The trees were five to seven meters high, had well-formed crowns and were in a good phytosanitary state (e.g. without lianas or parasitic plants). Fruits were haphazardly collected from throughout the canopy of each selected tree (Costa *et al.* 2010). All collected fruits were manually treated and using similarly sized seeds, with malformed seeds and those with visual signals of attack by predators or pathogens being eliminated. After processing, the seeds of all individuals were mixed and divided randomly among four treatments, with 100 seeds per treatment. The probability of germination was calculated assuming each seed to be a statistically independent experimental unit (see Warton & Hui 2011).

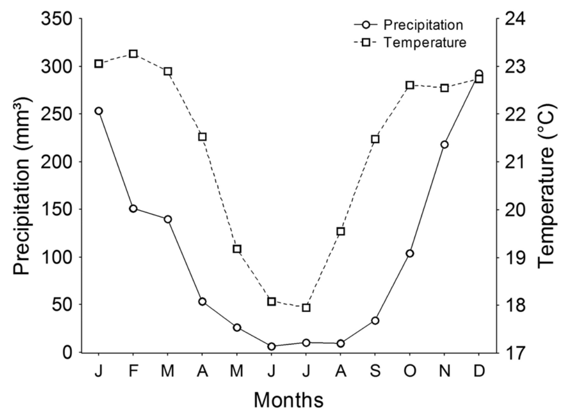

Figure 1. Average monthly precipitation and temperature in Paraopeba-MG.

The treatments used here simulate situations observed in the field that are suspected to influence the germination process. To evaluate the affect of aril removal by different seed dispersers and of fire on seed germination of *C. langsdorffii*, the seeds were submitted to the following treatments: control treatment, seeds placed to germinate with aril intact; acid treatment, simulation of the passage through the digestive tract of birds by exposing seeds to sulfuric acid (H_2SO_4) for 5 minutes; aril removed, simulation of aril removal by ants; fire treatment, seeds lacking arils into were partially buried in a layer of 5 cm of Cerrado soil in a 20 x 40 cm tray and covered by a litterfall layer from the study area, which was subsequently burned for about 30 minutes. After all treatments the seeds were rinsed with distilled water and tested for germination.

The seeds were placed in a gerbox, properly identified with their treatment, and covered with filter paper. The germination experiment was conducted in a germination chamber with controlled photoperiod and temperature (12 h/light at 30°C e 12 h/dark at 25°C). The seeds were monitored daily for 30 days to determine the percentage of germination and time to germination. A seed was considered germinated when primary root protrusion occurred (Ferreira & Borghetti 2004).

A soaking test was conducted under the same germination conditions using 30 different seeds. Seed mass was determined and then all seeds were immersed in distilled water and reweighed after 6, 18, 30, 48, 72, 96, 120, 144, 168, 192, 216 and 240 hours of water absorption. Relative increase in fresh weight (Wr) of seeds was calculated as $Wr = [(Wf - Wi)/ Wi] \times 100$ where Wi is the initial seed weight and Wf the weight after each time interval of water absorption (Baskin & Baskin 2004). Thus, imbibition curves were based on the increase in seed mass at different time intervals of seed immersion in distilled water.

Data Analysis

Data were analyzed using R software (R Core Team 2014). Germinability was evaluated by constructing a generalized linear model (GLM) using an appropriate error distribution for each response variable. The model was assessed via residual analysis (Crawley 2007). The germinability of a seed, based on a binary response (germinated or non germinated), is commonly expressed as a percentage (Ranal & Santana 2006). We used the binomial distribution error, indicated for binary data as the germination. Recently, Warton & Hui (2011) showed that this statistical approach provides a significant gain in power. The effect of treatments on germination was tested using the germination percentage of each treatment as response variables and treatments as explanatory variables.

To evaluate the probability of germination over a period of time, survival analysis was performed in order to test the effect of treatments on time of seed germination (Souza & Fagundes 2014). Thus, germination percentage within each treatment was used as response variables, while germination time was the explanatory variable. Survival analysis evaluates the likelihood of germination at a certain point in time, thus avoiding the temporal pseudo-replication inherent to the data.

Seed water absorption was tested by constructing Generalized Linear Mixed Models (GLMM), since these data also showed temporal pseudo-replication (Souza & Fagundes 2014). Thus, increased seed mass (Wr) was used as the response variable and time of water absorption (6, 18, 30, 48, 72, 96, 120, 144, 168, 192, 216 and 240 hours) as the explanatory variable.

Results

Germinability varied among treatments (X^2 = 89.735, *P*< 0.001). Our results demonstrated that aril removal positively influenced germination (Fig. 2). No significant differences were observed in the proportion of germinated seeds between acid treatment and without aril. No seed with aril intact (control treatment) germinated (Fig. 2). Fire also positively affected seed germination (Fig. 2), having the highest germination percentage among treatments with about 80% of the seeds germinating. At the end of the germination test, all non-germinated seeds were examined and found to be damaged and marked by the presence of fungi.

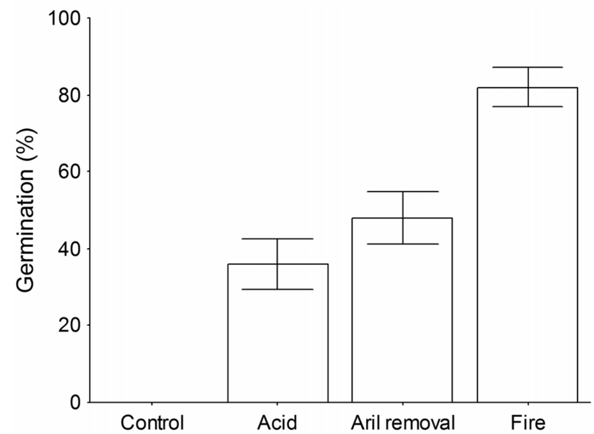

Figure 2. Percentage of seeds of *Copaifera langsdorffii* succesfully germinatiing in different treatments. Control: seeds placed to germinate with aril intact. Acid: simulation of the seed passage through the digestive tract of birds through exposing seeds to sulfuric acid for 5 minutes. Aril removal: simulation of aril removal by ants. Fire: aril removal followed by partial burial.

Overall, seeds germinated from the 4th to 25th day. The time to seed germination varied among treatments (X^2 = 16.225, *P*< 0.001). In this case, the removal of the aril affected germination time. Seeds submitted to acid treatment germinated more quickly than those without aril and those exposed to fire, which did not differ significantly (Fig. 3). The soaking test showed that water absorption varied with time (F = 243.609, P <0.001), and that the seeds of *C. langsdorffii* exhibited great variation in water imbibition (Fig. 4).

Discussion

In this study we examined some of the factors that affect seed germination of *Copaifera langsdorffii*. Our results demonstrate that removing the aril is essential for seed germination in this species, since the maintenance of the aril completely inhibited seed germination. Studies show

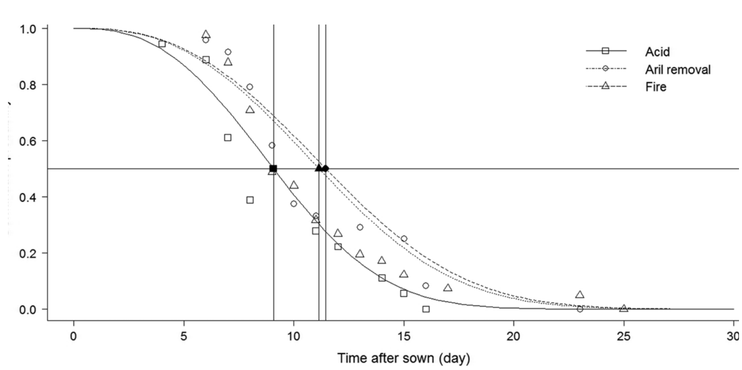

Figure 3. Time to seed germination for *Copaifera langsdorffii* in different treatments. Vertical lines indicate the time required for germination of 50% of the seeds. Acid: simulation of the seed passage through the digestive tract of birds by exposing seeds to sulfuric acid for 5 minutes. Aril removal: simulation of aril removal by ants. Fire: aril removal followed by partial burial.

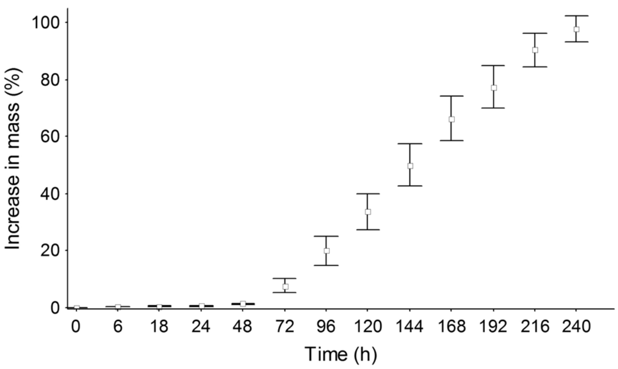

Figure 4. Imbibition curve for seeds of *Copaifera langsdorffii*.

that zoochorous seeds have secondary compounds that inhibit germination (Cipollini & Levey. 1997; Yagihashi & Miyamoto 1998; Robertson *et al.* 2006). The presence of secondary compounds (e.g. coumarins) in arils may inhibit seed germination by one of two ways: by direct chemical inhibition or by influencing micro-environmental factors such as light and oxygen (Cipollini & Levey 1997). The characteristics of the seeds of *C. langsdorffii* indicate that germination inhibition is chemical, since the seeds are not photoblastic and the aril only partially covers the seed. Furthermore, the presence of the aril favors the growth of fungi, which may prevent seed germination (Ohkawara & Akino 2005). Our results also show that the seeds of *C. langsdorffii* do not exhibit physical dormancy, despite their slow water uptake (Baskin & Baskin 2004).

Aril removal positively affected germination. The treatments that simulated the effects of a disperser increased germination by about 50%. These results are in accordance with other studies (Leal & Oliveira 1998; Robertson *et al.* 2006; Christianini *et al.* 2007; Lessa *et al.* 2013; Lima *et al.* 2013), and demonstrate the potential benefits of the seed dispersal process on seed germination (Lima *et al.* 2013). These results suggest that dispersers, in addition to transporting seeds away from the mother plant and thus avoiding intraspecific competition and decreasing the likelihood of an attack by predators (Janzen 1970; Swamy *et al.* 2011), are essential in the germination success of *C. langsdorffii*. Furthermore, during passage through the digestive tract of a frugivore, the seeds become scarified such that structures that can reduce or even prevent germination may be removed. Such scarification can accelerate the speed of germination and increase the proportion of successful germinating seeds (Robertson *et al.* 2006).

In many cases, zoochorous seeds, being produced in large numbers, are not all consumed by a primary disperser and usually fall to the soil near the mother plant, becoming available to other groups of animals, among which are other important dispersers (Christianini & Oliveira 2010; Lima *et al.* 2013). Specifically, *C. langsdorffii* has supra annual mass reproduction with high fruit production (Fagundes *et al.* 2013; Souza *et al.* 2015) and many seeds may go uneaten in this manner. Studies have shown that in such cases, ants are important secondary seed dispersers, carrying the seeds far from the mother plant (see Christianini & Oliveira 2010). Also, by removing the arils, ants reduce the chances of fungal attack on the seeds fallen on the fungi-prone tropical forest floor (Lima *et al.* 2013).

Our results indicate that fire was not detrimental to the survival of seeds of *C. langsdorffii*. Historically, fires appeared concomitantly with the origin of land plants and have played an important role throughout the history of life (Pausas & Keeley 2009). Traits adaptive to fire, such as tolerance to high temperatures, increased plant fitness in these environments (Keeley *et al.* 2011). Studies in savannas have shown that some species adapted to these environments are able to tolerate high temperatures from the passage of fire (Delgado *et al.* 2008; Fichino *et al.* 2012; Ribeiro *et al.* 2013; Ribeiro & Borghetti 2014). *Copaifera langsdorffii* is a common tree species in tropical forest environments (Carvalho 2003), and fire tolerance may be an attribute important for the occurrence of this species in the Cerrado (Rizzini 1976). Seeds of *C. langsdorffii* tolerate high temperatures, and the germination percentage increases after the passage of fire, which can be attributed to the control of microorganisms, such as fungi (Alencar *et al.* 2009). In fact, in our study, the seeds treated with fire had a low level of infestation by fungi.

Finally, our results show that biotic and abiotic factors can interact synergistically, affecting time to germination and the percentage of successful germinations of a zoochorous Neotropical tree. Evaluating the effects of dispersal on germination is important for understanding the qualitative effectiveness of seed dispersal, as well as elucidating the role that dispersers play in the population dynamics of plants (Schupp *et al* 2010). The lack of germination among seeds with their arils intact demonstrates the importance of dispersal to the germination of *C. langdorffii*. Thus, conservation of viable habitat for the maintenance of dispersers is essential for the reproductive success of *C. langsdoffii*. Considering the approaches, results and analyses of the present study, additional investigations into germination in field conditions are needed to elucidate the factors that determine the spatial distribution and abundance of species in natural plant communities.

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